

CLAIMS

1. A process for producing a culture fluid containing human thrombopoietin (hTPO), comprising the steps of:
culturing a eukaryotic cell expressing hTPO in a 3-6.5% serum-containing
5 medium;
subsequently culturing the cell in a 0.5-1.5% serum-containing medium;
and
culturing the cell in a serum-free medium that is substantially free from serum.
- 10 2. The process as set forth in claim 1, wherein the eukaryotic cell is a Chinese hamster ovary (CHO) cell line.
3. The process as set forth in claim 2, wherein the CHO cell line is selected from the group consisting of CHO dhfr-/pD40434 (KCTC 0630BP), CHO dhfr-/pD40449 (KCTC 0631BP) and CHO dhfr-/pD40458 (KCTC 0632BP).
- 15 4. The process as set forth in claim 1, wherein the eukaryotic cell is inoculated in the 0.5-1.5% serum-containing medium at a density of 1.0×10^4 to 1.0×10^6 cells/ml.
5. The process as set forth in claim 4, wherein the eukaryotic cell is inoculated at a density of 1.5×10^5 cells/ml.
- 20 6. The process as set forth in claim 1, wherein the serum-free medium is complemented with butyric acid and yeastolate.
7. A process for purifying human thrombopoietin (hTPO) from an hTPO-

containing biological fluid, comprising the steps of:

- (a) subjecting the biological fluid to affinity chromatography;
- (b) subjecting the eluate obtained at step (a) to hydrophobic interaction chromatography;
- 5 (c) subjecting the eluate obtained at step (b) to reverse phased chromatography; and
- (d) subjecting the eluate obtained at step (c) to anion exchange chromatography.

10 8. The process as set forth in claim 7, wherein the eluate obtained at step (c) is loaded onto an ionic exchange chromatography column, and hTPO eluted selectively from the column by a 0.15-0.3M sodium chloride gradient is collected.

9. The process as set forth in claim 7, further comprising a step of carrying out gel filtration chromatography after step (d).

15 10. The process as set forth in claim 7, wherein the hTPO-containing biological fluid is a culture supernatant from the culture fluid produced by the process of the claim 1.

11. The process as set forth in claim 7, wherein a column used in the affinity chromatography at step (a) is eluted with phosphate buffer containing 1 M sodium chloride.

20 12. The process as set forth in claim 7, wherein a column used in the reverse phased chromatography at step (c) is eluted with an ethanol gradient.

13. A fraction containing hTPO purified by the process of claim 8.